year frogs [8]. This mode of appearance coincides well with the increasing change in the plasma concentrations of testosterone and DHT. It is known that testosterone is capable of conversion to DHT or estrogen, but DHT is not capable of further change and is an androgen with the strongest physiological activities [16, 17]. In maturing male frogs, the levels of plasma DHT were always higher than those of testosterone, whereas in subadult and adult female frogs, only testosterone fluctuated, and DHT remained at a constant low level except for a peak which appeared in the second-year June frogs. It is known that in adult males of some *Rana* species DHT is a major component of serum androgens [3, 18–20]. Iwasawa and Kobayashi [21] reported that the administration of testosterone and estradiol to first-year frogs induced considerable development of both Wolffian and Müllerian ducts. These results suggest that a good deal of androgen detected in second-year late spring male frogs and early summer female frogs has a stimulative effect on the development of genital tracts.

Role of plasma androgens in female frogs

In the present study, the plasma testosterone levels in female frogs were not so low as compared with those in male ones, except for second- and third-year male frogs in autumn when the concentration of plasma androgen showed a marked increase. In *R. esculenta* [1] and *R. catesbeiana* [3] also, the concentrations of testosterone in the serum of adult female frogs are almost the same, or even greater than those in adult male ones, and the levels of testosterone are higher than those of estradiol.

Delrio *et al.* [22] showed that testosterone and DHT were produced in the ovaries of *R. esculenta.* Fortune and Tsang [23] reported that the ovaries of *Xenopus laevis* produced a large amount of testosterone after treatment with frog pituitaries. Habbard and Licht [24] found, in *R. pipiens* and *R. catesbeiana*, that gonadotropin stimulated the secretion of testosterone and progesterone in the ovaries, and oocyte maturation occurred simultaneously. Licht *et al.* [3] reported that the plasma androgen levels in adult females were highly correlated to the developmental condition of the Müllerian ducts. A similar phenomenon was observed in subadult female frogs of *R. nigromaculata* [8]. Considering these facts together with the results of Iwasawa and Kobayashi [21], it is conceivable that the androgens found in the serum of female *R. nigromaculata* are produced by the ovaries, and that the serum testosterone in the female frogs acts not only as the precursor estrogens [22], but acts stimulatively on the sex organs as an androgen.

We think that the number of adult frogs used in the present study is not enough to clarify the pattern of seasonal changes in androgen levels compared with other anuran species, so we have not referred to the problem in this paper.

REFERENCES

1 D'Istria, M., Delrio, G., Botte, V. and Chieffi, G. (1974) Radioimmunoassay of testosterone,17β-oestradiol and oestrone in the male and female plasma of *Rana esculenta* during sexual cycle. Steroids Lipids Res., **5**: 42–48.

2 Siboulet, R. (1981) Variations saisonnieres de la teneur plasmatique en testostérone et dihydrotestostérone chez le crapaud de Mauritanie (*Bufo mauritanicus*). Gen. Comp. Endocrinol., **43**: 71–75.

3 Licht, P., McCreery, B. R., Barnes, R. and Pang, R. (1983) Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana.* Gen. Comp. Endocrinol., **50**: 124–145.

4 Kera, Y. and Iwasawa, H. (1981) Functional histology of the testies in the process of sexual maturation in the frog, *Rana nigromaculata.* Zool. Mag. (Tokyo), **84**: 109–114. (in Japanese with English summary)

5 Iwasawa, H. and Asai, O. (1959) Histological observation on the seasonal change of the testis and the thumb pad in the frog, *Rana nigromaculata.* J. Fac. Sci. Niigata Univ., Ser. II, **2**: 213–219.

6 Iwasawa, H. and Kobayashi, M. (1976) Development of the testis in the frog *Rana nigromaculata*, with special reference to germ cell maturation. Copeia, **1976**: 461–467.

7 Iwasawa, H. and Kera, Y. (1982) Structural change in gonadotropin-producing cells of male frogs, *Rana nigromaculata*, in the process of sexual maturation. Gen. Comp. Endocrinol., **46**: 236–245.

8 Iwasawa, H., Nakazawa, T. and Kobayashi, T. (1987) Histological observations on the reproductive organs of growing *Rana nigromaculata* frogs. Sci. Rep. Niigata Univ. Ser. D, **24**: 1–13.

9 Ismail, A. A. A., Niswender, G. D. and Midgley,

A. R. (1972) Radioimmunoassay of testosterone without chromatography. J. Clin. Endocrinol. Metab., **34**: 177–184.

10 Greenwood, F. C. and Hunter, W. M. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J., **89**: 114–123.

11 Lofts, B. (1974) Reproduction. In "Physiology of the Amphibia". Ed. by B. Lofts, Academic Press, New York, vol. 2, pp. 107–218.

12 Lofts, B. (1984) Amphibians. In "Marshall's Physiology of Reproduction". Vol. 1, Ed. by G. E. Lamming, Churchill Livingstone, Edinburgh, pp. 127–205.

13 Licht, P. (1979) Reproductive endocrinology of reptiles and amphibians: gonadotropins. Ann. Rev. Physiol., **41**: 337–351.

14 Muller, C. H. and Licht, P. (1980) Gonadotropin specificity of androgen secretion by amphibian testes. Gen. Comp. Endocrinol., **42**: 365–377.

15 Kobayashi, T. and Iwasawa, H. (1986) Effects of testosterone on spermatogenic process and sperm preservation in late autumn young *Rana nigromaculata*. Zool. Sci., **3**: 387–390.

16 Gower, D. B. and Fotherby, K. (1975) Biosynthesis of the androgens and oestrogens. In "Biochemistry of Steroid Hormones". Ed. by H. L. J. Makin, Blackwell Sci. Publ., Oxford, pp. 77–104.

17 Gower, D. B. (1984) Biosynthesis of the androgens and C_{19} steroids. In "Biochemistry of Steroid Hormones". Ed. by H. L. J. Makin, Blackwell Sci. Publ., Oxford, pp. 170–206.

18 Wada, M., Wingfield, J. C. and Gorbman, A. (1976) Correlation between blood level of androgens and sexual behavior in male leopard frogs, *Rana pipiens*. Gen. Comp. Endocrinol., **29**: 72–77.

19 Muller, C. H. (1976) Steroidogenesis and spermatogenesis in the male bullfrog, *Rana catesbeiana*: Regulation by purified bullfrog gonadotropins. Ph. D. Dissertation, Univ. California, Berkeley.

20 Muller, C. H. (1977) Plasma 5α-dihydrotestosterone and testosterone in the bullfrog, *Rana catesbeiana:* Stimulation by bullfrog LH. Gen. Comp. Endocrinol., **33**: 122–132.

21 Iwasawa, H. and Kobayashi, M. (1974) Effect of testosterone and estradiol on the development of sexual characters in young *Rana nigromaculata*. Biol. Reprod., **11**: 398–405.

22 Delrio, G., D'Istria, M., Iela, L. and Chieffi, G. (1979) The possible significance of testosterone in the female green frog *Rana esculenta* during sexual cycle. Boll. Zool., **46**: 1–9.

23 Fortune, J. E. and Tsang, P. C. (1981) Production of androgen and estradiol-17β by *Xenopus* ovaries treated with gonadotropins *in vitro*. Gen. Comp. Endocrinol., **43**: 234–242.

24 Hubbard, G. M. and Licht, P. (1986) *In vitro* ovarian responses to pulsatile and continuous gonadotrophin administration on steroid secretion and oocyte maturation in the frogs, *Rana pipiens* and *Rana catesbeiana*. Gen. Comp. Endocrinol., **61**: 417–423.

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Vascular Supply of Hypophysis in the Turtle, *Geoclemys reevesii*

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ABSTRACT—The vascular route in the hypothalamo-hypophysial complex was studied histologically in the turtle, *Geoclemys reevesii.* Distinct anterior and posterior groups of portal vessels originating from the anterior and posterior capillary plexus of the median eminence have been demonstrated. The anterior group of portal vessels breaks up into capillaries in the cephalic lobe of the pars distalis, whereas the posterior group of portal vessels is continuous with a capillary network in the caudal lobe of the pars distalis. It is suggested that the presence of two distinct groups of vascular routes is correlated with the cytological differentiation of the pars distalis.

INTRODUCTION

The vascularization of the hypothalamus and hypophysis has been described in detail for various vertebrate species. The reptilian hypophysial portal system has been investigated previously by Enemar in the lizard and the snake [1], by Green in the turtle and several other species [2], by Wingstrand in the snake [3]. Such studies have demonstrated the significance of the hypophysial portal system by which hypophysiotropic neurohormones may be transported from the median eminence (ME) into the pars distalis (PD). In the previous studies on the vascularization of the hypophysis of the turtle, *Pseudemys scripta*, the presence of two distinct anterior and posterior groups of capillary plexus and portal vessel system arising from the anterior and posterior primary capillary plexus of the anterior and posterior divisions of the ME has been estimated briefly [4].

Reported here are the precise results of an investigation designed to ascertain whether the division of portal vessels into anterior and posterior groups is present in the turtle, *Geoclemys reevesii.* Since there is clear differential distribution of the PD cells, the correlation between the vascular supply and the cytological differentiation of the PD cells is also examined.

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MATERIALS AND METHODS

Adult male and female turtles were obtained from a dealer. The hypothalamic regions of 10 animals were fixed in Bouin's solution and prepared for routine histology. Thin paraffin sections of 6 μm were stained with Heidenhain's Azan technique or Gomori's paraldehyde fuchsin (AF). For the study of the vascularization, 8 animals were injected with filtered India ink-saline solution (1:1) through the ventricle of the pulsating heart or into the left carotid artery. After decapitation, the brains were fixed in Bouin's solution. Subsequently they were trimmed to small pieces. Thick paraffin sections of 100 μm were prepared without any staining procedure.

RESULTS

In the turtle, the ME forms a well-developed swelling on the ventral side of the infundibulum and can be divisible into anterior and posterior regions (Fig. 1). Both regions of the ME are supplied with distinct components of fibers from different areas of the hypothalamus, preoptic hypothalamic, and tuberal regions. The anterior ME receives a rich supply of fibers containing AF-stainable neurosecretory material from the

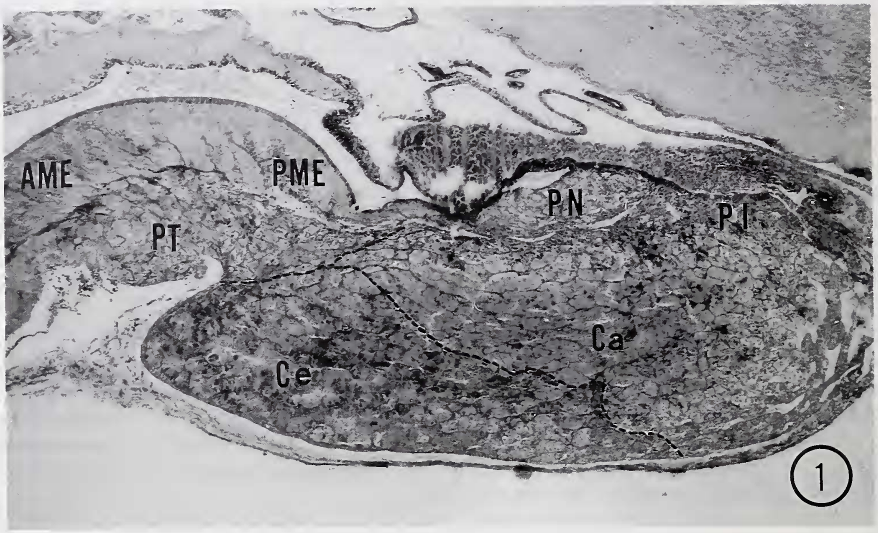

FIG. 1. Mid-sagittal section through the infundibulum and hypophysis. AME, anterior ME; Ca, caudal lobe of PD; Ce, cephalic lobe of PD; PI, pars intermedia; PME, posterior ME; PN, pars nervosa; PT, pars tuberalis. Dotted line represents the boundary between Ce and Ca. AF stain. ×60.

anterior hypothalamus, whereas the posterior ME receives only a few fibers of neurosecretory nature.

The ME is covered by a very dense primary capillary plexus deriving from branches of the infundibular arteries. Some of the capillaries penetrate deeply into the palisade layer of the ME. As shown in Figure 2, there are two distinct anterior and posterior capillary plexus, corresponding to the anterior and posterior regions of the ME, respectively.

At the mid-ventral surface of the ME, these two groups of capillary plexus converge into two groups, anterior and posterior, of portal vessels. The vascular connections between the capillary plexus of the ME and the portal vessels can be demonstrated in transverse sections of the India ink-injected specimens (Fig. 3).

Several large portal vessels pass vertically downward from the primary capillary plexus of the anterior ME, and break up into a secondary sinusoidal-capillary net within the anterior portion of the PD, cephalic lobe. Similarly, the capillaries of the posterior ME converge to form large portal vessels leading to the secondary sinusoidal-capillary net within the posterior region of the PD, caudal lobe (Figs. 2 and 3).

The elongated PD consists of well-defined cephalic and caudal lobes, which are distinct in their cellular constituents (Fig. 1). At least four types of secretory cells are characterized by the cell size and tinctorial affinities for specific dyes. The regional patterns of the cellular distribution are demonstrable in the two lobes. The cephalic lobe is occupied by abundant carminophilic acidophils and amphophils, whereas the caudal lobe contains orangeophilic acidophils and basophils (Fig. 4). The capillaries in the cephalic and caudal lobes are almost independent of each other and form a dense network around the secretory cells of cephalic and caudal lobe, respectively.

DISCUSSION

The vascularization of the turtle hypophysis has been investigated previously, and the presence of portal system which supplies the PD with venous blood from the capillaries of the primary capillary plexus in the ME has been demonstrated [2, 5–8]. The primary capillary plexus of the ME arising from the branches of infundibular arteries is well developed and the blood supply to the hypophysis is fundamentally the same as in the other reptilian species [9]. Although there are species differences, these previous studies reveal that the portal

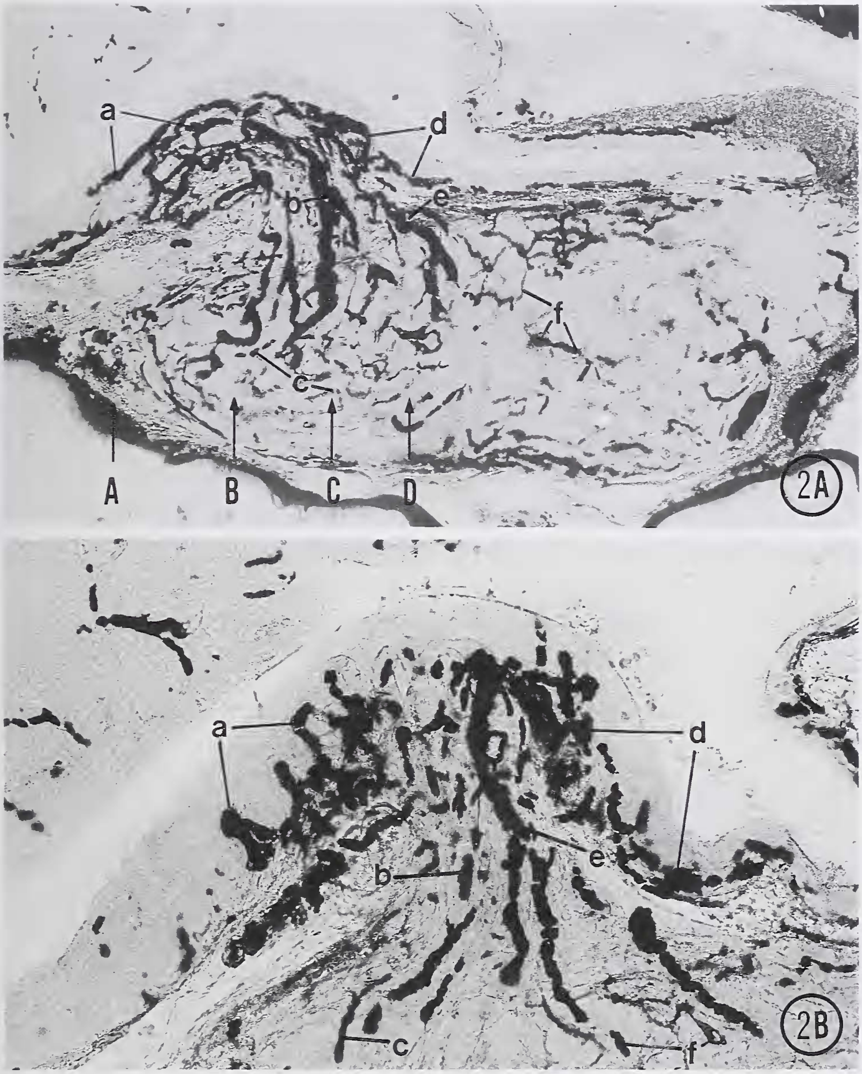

FIG. 2. Mid-sagittal section through the infundibulum and hypophysis of India ink-injected specimen (A). Note the two groups of primary capillary plexus and the portal vessels (B). a, primary capillary plexus in the anterior ME; b, anterior group of portal vessels; c, secondary capillary plexus in the cephalic lobe; d, primary capillary plexus in the posterior ME; e, posterior group of portal vessels; f, secondary capillary plexus in the caudal lobe; Arrows with A–D represent the transverse planes of the sections in Fig. 3. A, ×45; B, ×77.

vessels collect the effluent blood from the primary capillary plexus of the ME and pass vertically downward to the PD.

The present study indicates that the ME has distict anterior and posterior regions and is covered by a well developed capillary network, corresponding to the anterior and posterior capillary plexus. Moreover, there are distinguishable anterior and posterior groups of portal vessels originating from the anterior and posterior capillary plexus of the ME, respectively. These observations suggest that there are regional specialization of the vascular system corresponding to the drainage of the ME capillary blood containing hypothalamic neurosecretory factors. The existence of regional specialization of the vascular system in the ME is

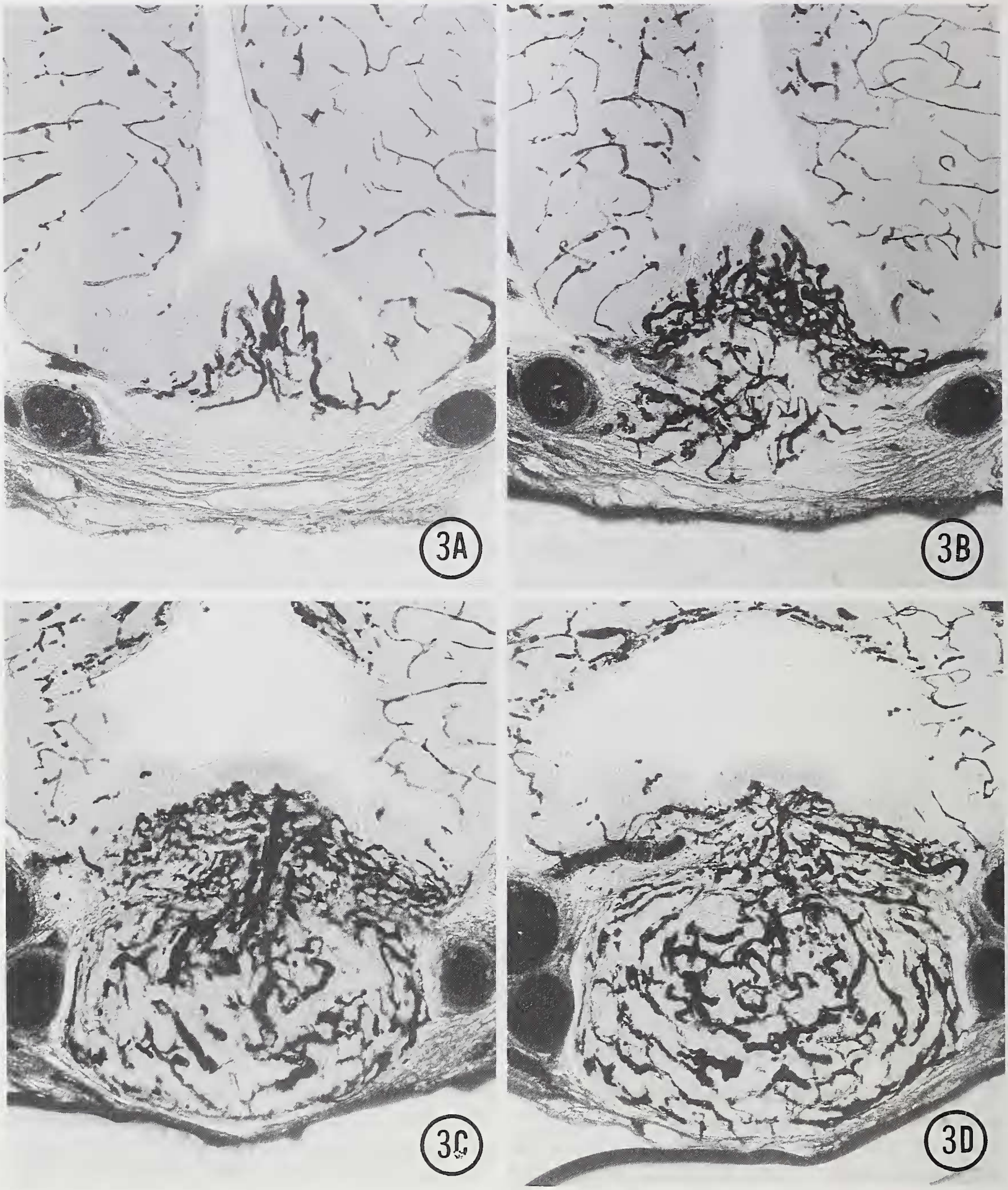

FIG. 3. Transverse sections through the infundibulum and hypophysis of India ink-injected specimen. Primary capillary plexus penetrating into the anterior ME is indicated in A. Secondary capillary plexus in the cephalic lobe occupies the bottom of B. Anterior group of portal vessels can be noted in C. Primary capillary plexus in the posterior ME and secondary capillary plexus in the caudal lobe are indicated in D. A–D, ×45.

in good accord with previous findings in the turtle, *Pseudemys scripta* [4]. In the turtle, *Chrysemys picta*, the primary capillary net forming a dense plexus in the ME does not show any anatomical division into anterior and posterior plexus [2].

Holmes and Ball [10] have demonstrated that the reptilian ME is divisible into anterior and posterior regions, as in birds. Using electron microscopy, structural difference between the anterior and posterior ME has been reported in